

1 Claims

2

3 1. An expression vector comprising DNA encoding a
4 subunit of a dimeric form of interleukin under
5 transcriptional control of an ecdysone-inducible
6 promoter.

7

8 2. A vector as claimed in Claim 1 in which the
9 subunit of a dimeric form of interleukin is selected
10 from the group comprising: p35 (alpha) subunit of
11 interleukin 12 (IL-12); p40 (beta) subunit of IL-12;
12 p19 chain of IL-23; p40 subunit of IL-23; ebi3
13 subunit of IL-27; and p28 subunit of IL-27.

14

15 3. A vector as claimed in Claim 1 or 2 comprising
16 an ecdysone-inducible mammalian expression plasmid,
17 wherein the DNA encoding the subunit of a dimeric
18 form of interleukin is included in the plasmid.

19

20 4. A vector as claimed in any preceding Claim in
21 which the DNA encodes a p40 subunit of IL-12.

22

23 5. A vector as claimed in any of Claims 1 to 3 in
24 which the DNA encodes a p35 subunit of IL-12.

25

1 6. A vector as claimed in any of Claims 1 to 3 in
2 which the DNA encodes a p19 subunit of IL-23.

3

4 7. An expression vector as claimed in Claim 1 or
5 6 in which the ecdysone inducible mammalian
6 expression vector is selected from the group
7 comprising: pIND; pIND(SP1); and pINDHygro.

8

9 8. An expression vector as claimed in any of
10 Claims 1 to 7 in which the DNA encoding a subunit of
11 dimeric interleukin 12 includes a DNA sequence
12 encoding a 6 x histidine tag.

13

14 9. An expression vector as claimed in any
15 preceding Claim selected from the group comprising:
16 pIND-p35H; pIND(SP1)-p35H; pIND-40H; pINDHygro-p40;
17 pIND(SP1)-p40H; and pIND-p40.

18

19 10. An expression vector as claimed in any
20 preceding Claim in which the DNA encoding the subunit
21 of dimeric interleukin is digested with NheI and XhoI
22 restriction enzymes prior to ligation of the digested
23 DNA products into the expression vector.

24

25 11. The expression vector pIND(SP1)-p35H having
26 ECACC accession number 03120401.

27

28 12. A method a producing a tightly controlled
29 expression vector capable of transforming a host cell
30 which when transformed is capable of producing a

1 recombinant dimeric interleukin, or a subunit
2 thereof, under transcriptional control of an
3 ecdysone-inducible promoter, comprising the steps of:
4 - providing cDNA for a subunits of a dimeric
5 interleukin;
6 - digesting the cDNA with at least one restriction
7 enzyme; and
8 - ligating the digested cDNA product into an
9 ecdysone-inducible mammalian expression vector.

10

11 13. A method as claimed in Claim 12 in which the
12 one or more restriction enzymes consist of *NheI* and
13 *XhoI*.

14

15 14. A method as claimed in Claim 12 or 13 in which
16 the ecdysone-inducible mammalian expression vector is
17 selected from the group comprising: pIND; pIND(SP1);
18 and pINDHygro.

19

20 15. A method as claimed in any of Claims 12 to 14
21 in which the cDNA for the subunit of dimeric
22 interleukin includes a DNA sequence encoding a 6 x
23 histidine tag.

24

25 16. An expression vector obtainable by the method
26 of any of Claims 12 to 15.

27

28 17. A cell line transfected with at least one
29 expression vector of any of Claims 1 to 11 or 16,
30 wherein the DNA encoding the at least one subunit of

1 a dimeric interleukin is under the transcriptional
2 control of an ecdysone-inducible mammalian expression
3 system.

4

5 18. A cell line according to Claim 17 and capable
6 of producing homodimeric IL-12, the cell line being
7 transfected with an expression vector of Claim 4.

8

9 19. A cell line according to Claim 17 and capable
10 of producing heterodimeric IL-12, the cell line being
11 transfected with an expression vector of Claim 4 and
12 an expression vector of Claim 5.

13

14 20. A cell line according to Claim 17 and capable
15 of producing heterodimeric IL-23, the cell line being
16 transfected with an expression vector of Claim 4 and
17 an expression vector of Claim 6.

18

19 21. A cell line of any of Claims 17 to 20 which
20 includes a plasmid pVgRxR.

21

22 22. A cell line as claimed in any of Claims 17 to
23 21 in which the cells are human embryonic kidney
24 cells.

25

26 23. A cell line as claimed in Claim 22 in which
27 the cells are EcR293 cells.

28

1 24. A cell line as claimed in any of Claims 17 to
2 20 in which the cells are natural β subunit-producing
3 cells such as a HIBERNIA1 cell line.

4
5 25. A cell line having ECACC accession number
6 03112701.

7
8 26. A method of producing a cell line capable of
9 producing a recombinant dimeric interleukin, or a
10 subunit thereof, under transcriptional control of an
11 ecdysone-inducible promoter, comprising the steps of:
12 - providing at least one expression vector
13 according to any of Claims 1 to 11 or 16; and
14 - transfecting a host cell with the at least one
15 expression vector,
16 - wherein the DNA encoding the at least one
17 subunit of a dimeric interleukin is under the
18 transcriptional control of an ecdysone-inducible
19 mammalian expression system.

20
21 27. A method of preparing cDNA encoding a subunit
22 of a dimeric form of interleukin comprising the steps
23 of providing cDNA encoding the subunit, and digesting
24 the cDNA with restriction enzymes *NheI* and *XhoI* to
25 obtain a cDNA product.

26
27 28. A method of screening a candidate compound for
28 the ability to inhibit dimer assembly and secretion
29 of a dimeric form of interleukin, comprising the
30 steps of:

- 1 - incubating a cell culture comprising a cell line
- 2 of any of Claims 17 to 25 with the candidate
- 3 compound;
- 4 - inducing transcription of the dimeric
- 5 interleukin in the cells of the culture using
- 6 ecdysone or an ecdysone analog; and
- 7 - assaying the cell culture for the presence of
- 8 secreted interleukin.

9

10 29. A method as claimed in Claim 28, and in which
11 the interleukin expressed by the cell line has a 6 x
12 histidine amino acid sequence tagged on either or
13 both of the subunits thereof, wherein the assaying
14 step involves Ni-NTA affinity chromatography.

15

16 30. A method as claimed in Claim 28 in which the
17 assaying step involves probing the cell culture with
18 an antibody specific to a dimeric form of
19 interleukin, or a subunit thereof.

20

21 31. An inhibitor of dimer assembly and secretion
22 of dimeric interleukin identified by the method of
23 any of Claims 28 to 30.

24

25 32. A method of prevention or treatment of
26 inflammatory disease comprising a step of treating an
27 individual with an inhibitor of Claim 31.

28

29 33. A method of treating disease having a
30 pathogenesis which includes endogenous production of

1 any of cytokines IL-12, IL 23 or IL-27, the method
2 comprising a step of treating an individual with an
3 endoplasmic reticulum (ER) Ca^{2+} perturbation reagent.
4

5 34. Use of an ER Ca^{2+} perturbation reagent in the
6 manufacture of a medicament for the treatment of
7 disease having a pathogenesis which includes
8 endogenous production of any of cytokines IL-12, IL-
9 23 or IL-27.
10

11 35. Use of an ER Ca^{2+} perturbation reagent for the
12 treatment of disease having a pathogenesis which
13 includes endogenous production of any of cytokines
14 IL-12, IL-23 or IL-27.
15

16 36. A method of inhibiting the formation of one or
17 more cytokines in an individual, which method
18 comprises the step of treating an individual with ER
19 Ca^{2+} perturbation reagent.
20

21 37. Use of an ER Ca^{2+} perturbation reagent to
22 inhibit the formation of one or more cytokines in an
23 individual.
24

25 38. A method or use as claimed in any of Claims 33
26 to 37 in which the disease is an inflammatory disease
27 in which one or more endogenously produced IL-12
28 forms play a disease promoting role.
29

1 39. A method or use as claimed in Claim 38 in
2 which the IL-12 forms are $\alpha\beta$ heterodimeric and $\beta\beta$
3 homodimeric forms.

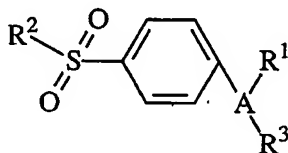
4
5 40. A method or use as claimed in any of Claims 33
6 to 39 in which the disease is selected from the group
7 consisting of infectious diseases; bacterial
8 protozoal or virus-induced inflammation; epithelial
9 airway inflammation such as asthma; allergic disease;
10 autoimmune disease such as MS, RA and Inflammatory
11 Bowel Disease; and -all conditions in which
12 endogenously produced IL-12 α/β or $\beta\beta$ forms are
13 thought to play a disease-promoting role.

14
15 41. A method or use as claimed in any of Claims 33
16 to 40 in which the ER Ca^{2+} perturbation reagent is
17 selected from the compounds of Formula I:

18

19

20 Formula I



21

22 wherein A is a substituent selected from partially
23 unsaturated or unsaturated heterocyclyl and partially
24 unsaturated or unsaturated carbocyclic rings;
25 wherein R^1 is at least one substituent selected from
26 heterocyclyl, cycloalkyl, cycloalkenyl and aryl,

1 wherein R¹ is optionally substituted at a
2 substitutable position with one or more radicals
3 selected from alkyl, haloalkyl, cyano, carboxyl,
4 alkoxycarbonyl, hydroxyl, hydroxyalkyl, amino,
5 alkylamino, arylamino, nitro, alkoxyalkyl,
6 alkylsulfinyl, halo, alkoxy and alkylthio;
7 wherein R² is methyl or amino; and
8 wherein R³ is a radical selected from hydrido, halo,
9 alkyl, alkenyl, oxo, cyano, carboxyl, cyanoalkyl,
10 heterocycloxy, alkyloxy, alkylthio, alkylcarbonyl,
11 cycloalkyl, aryl, haloalkyl, heterocyclyl,
12 cycloalkenyl, aralkyl, heterocyclalkyl, acyl,
13 alkylthioalkyl, hydroxyalkyl, alkoxycarbonyl,
14 arylcarbonyl, aralkylcarbonyl, aralkenyl,
15 alkoxyalkyl, arylthioalkyl, aryloxyalkyl,
16 aralkylthioalkyl, aralkoxyalkyl, alkoxyaralkoxyalkyl,
17 alkoxycarbonalkyl, aminocarbonyl, aminocarbonylalkyl,
18 alkylaminocarbonyl, N-arylaminocarbonyl, N-alkyl-N-
19 arylaminocarbonyl, alkylaminocarbonylalkyl,
20 carboxyalkyl, alkylamino, N-arylamino, N-
21 aralkylamino, N-alkyl-N-aralkylamino, N-alkyl-N-
22 arylamino, aminoalkyl, alkylaminoalkyl, N-
23 arylaminoalkyl, N-aralkylaminoalkyl, N-alkyl-N-
24 aralkylaminoalkyl, N-alkyl-N-arylaminalkyl, aryloxy,
25 aralkoxy, arylthio, aralkylthio, alkylsulfinyl,
26 alkylsulfonyl, aminosulfonyl, alkylaminosulfonyl, N-
27 arylaminosulfonyl, arylsulfonyl, N-alkyl-N-
28 arylaminosulfonyl; or a pharmaceutically-acceptable
29 salt thereof.